Potassium Nitrate-Toxicity & Teratogenicity Studies in Avian Embryos-FDA Contract #71-330 No Date

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POTASSIUM NITRATE

Toxicity and Teratogenicity Studies in Avian Embryos

FDA Contract #71-330

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Studies on the Toxicity and Teratogenicity of Potassium Nitrate in Avian Embryos

SUMMARY and CONCLUSIONS

Potassium nitrate was embryo toxic when injected into the air cell of chicken eggs at either 96 hours incubation or prior to incubation. The estimated LD-50 value for air cell 0 hours was 569 mg/kg with a 95% confidence range of 460 to 775 mg/kg; the value estimated for air cell 96 hours was 73 mg/kg with a range of 33-151. Potassium nitrate was about 8 times more toxic to 96 hour embryos.

Significant increases in H-S-L-V abnormalities in the embryos were not obtained for any of the protocols suggesting that potassium nitrate was not teratogenic under the conditions of these studies.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #71-330 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (post-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range- finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

The remaining birds in each group were maintained to 6 months of age and then sacrificed.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
   a. % response against dose
   b. % response against log dose
   c. log % response against dose
   d. arcsin transformation against dose
   e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
   a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Potassium was solubilized in distilled water for use in the test protocols. Air cell administrations were carried out with dose levels of 10 to 1000 mg/kg, while yolk injections were accomplished using levels of 1.0 to 320 mg/kg.

RESULTS and DISCUSSION

Mortality - the percentage mortalities obtained for the test protocols are shown in Tables 1 - 4. When potassium nitrate was administered in the air cell prior to incubation (Table 1) significant increases in mortality were obtained at dose levels of 500 and 1000 mg/kg (Table 5). Potassium nitrate was more toxic to the embryo when injected into the air cell after 96 hours of development (Table 2). Dose levels of 20 mg/kg and above showed significantly ($P \geq 0.05$) more mortality than the water injected control eggs (Table 5).

Yolk administrations of the compound showed increased mortalities for only the zero time with dose levels of 1.0 and 100.0 mg/kg (Tables 3, 4 & 5).

Linear Regression analyses of log of dose level against the probit of mortality indicated a significant relationship for only the air cell administration route. The resulting regression equations predicted LD-30, 50, 70 and 90 values with a 95% confidence range as shown in Table 6. Potassium nitrate was considerably more toxic to avian embryos at 96 hours than when administered prior to incubation.

Teratology - The incidence of abnormalities in the four test protocols are summarized in Tables 1 - 4. Statistical evaluations of these data by chi-square analyses suggest a significant ($P \geq 0.05$). increase in abnormalities in the embryos for only those injected with the test substance at 30.0 and 200 mg/kg in the air cell at zero hours incubation (Table 7). These significant chi-square values resulted from the histopathological findings in these two groups (Table 9). The histological findings showed liver fibrosis and vacuolization along with kidney tubular granulation.

The occurrence of abnormalities upon gross examination of head, limbs, skeleton and viscera was not significantly different from that of the solvent control embryos (Table 8).

These data suggest that although potassium nitrate was toxic to chicken embryos; gross teratogenic findings were negative. The significant incidence of histopathological findings of liver and kidney involvement suggest the need for futher investigation.

Post-Hatch Data - Chicks having received either 0, 10.0 or 320.0 mg potassium nitrate per kg were saved for six months (Table 10). Hatch weight appeared to be slightly decreased for those chicks which had received the test substance, but this was not apparent in the six-week body weights or at six months (Table 10). Feed consumption data do not indicate that potassium nitrate affected appetite.

TABLE 1

K Nitrate in H_2O

Summary

Air Cell - 0 Hrs.

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Toxic Response %	Toxic Response #	Functional %	Functional #
000.0	20	70.00	14	0																	
500.0	19	52.63	10	0																	
320.0	99	20.20	20	0																	
200.0	45	8.88	4	20.00	9	2.22	1	2.22	1												
100.0	9	22.22	2	0																	
80.0	100	9.00	9	0																	
40.0	99	3.03	3	1.01	1	1.01	1	1.01	1												
30.0	36	2.77	1	19.44	7																
20.0	117	11.11	13	0.85	1													0.85	1		
10.0	119	10.92	13	1.68	2	0.84	1							0.84	1					0.84	1
0.0	241	10.78	26	0.41	1	0.41	1	0.41	1												
rilled	359	10.02	36	0.27	1	0.27	1					0.27	1								
reated	461	3.25	15	0.86	4	0.65	3	0.43	2					0.21	1					0.43	2

SUMMARY - ALL DOSE LEVELS

	663	13.42	89	3.0	20	0.45	3	0.30	2					0.15	1			0.15	1	0.15	1

TABLE 2

K Nitrate in H_2O

Summary

Air Cell - 96 Hrs.

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Response %	Response #	Functiona %	Functiona #
700.0	27	92.59	25																		
350.0	27	96.29	26	0																	
320.0	115	98.26	113	0																	
175.0	27	66.66	18	0																	
160.0	50	78.00	39	0																	
88.0	27	51.85	14	0																	
80.0	114	68.70	79	0																	
40.0	114	53.0	61	0																	
20.0	142	6.33	9	0.70	1	0.70	1					0.70	1								
10.0	65	1.53	1	0																	
0.0	221	0		0																	
illed	246	2.84	7	0																	
reated	461	3.25	15	0.86	4	0.65	3	0.43	2							10.21	1			0.43	

SUMMARY - ALL DOSE LEVELS

836	54.53	459	0.12	1	0.12	1			0.12	1			

TABLE 3

K Nitrate in H_2O

Summary

Yolk - 0 Hrs

Dose, ppm	No. Fertile	Mortality %	#	Abnormal Total %	#	Abnormal H-S-V-L %	#	Abnormalities by category: Head %	#	Skeletal %	#	Viscera %	#	Limbs %	#	Struc-tural %	#	Response %	#	Functiona %	#
20.0	10	60.00	6	0																	
00.0	144	32.63	47	1.38	2	1.38	2	1.38	2												
00.0	13	69.23	9	0														0.71	1		
30.0	139	28.77	40	0.71	1															0.65	
40.0	152	24.34	37	1.97	3	1.31	2					1.31	2								
20.0	138	27.53	38	0.72	1											0.72	1				
0.0	124	23.38	29	1.61	2	1.61	2	0.80	1			0.80	1								
1.0	42	85.71	36	2.38	1	2.38	1	2.38	1												
0.0	140	33.57	47	0																	
ierced	30	13.33	4	3.33	1	3.33	1	3.33	1												
treated	461	3.25	15	0.86	4	0.65	3	0.43	2					0.21	1					0.43	

SUMMARY - ALL DOSE LEVELS

762	31.76 242	1.31 10	0.92 7	0.52 4	0	0.39 3	0	0.13 1	0.13 1	0.13

TABLE 4

K Nitrate in H_2O

Summary

Yolk - 96 Hrs

Dose, ppm	No. Fertile	Mortality %	Mortality #	Abnormal Total %	Abnormal Total #	Abnormal H-S-V-L %	Abnormal H-S-V-L #	Abnormalities by category: Head %	Head #	Skeletal %	Skeletal #	Viscera %	Viscera #	Limbs %	Limbs #	Structural %	Structural #	Response %	Response #	Functional %	Functional #
0.0	90	10.00	9	0																	
0.0	29	20.68	6	0																	
0.0	120	9.16	11	1.66	2	0.83	1	0.83	1												
0.0	120	9.16	11	2.50	3	0.83	1	0.83	1											0.83	1
0.0	120	14.16	17	1.66	2	0.83	1	0.83	1									0.83	1		
0.0	120	13.33	16	0.83	1	0.83	1	0.83	1												
0.0	120	10.00	12	0.83	1	0.83	1	0.83	1												
rced	240	9.16	22	0.83	2	0.83	2	0.83	2												
reated	461	3.25	15	0.86	4	0.65	3	0.43	2					0.21	1					0.43	2

SUMMARY - ALL DOSE LEVELS

599	11.69	70	1.34	8	0.67	4	0.67	4					0.17	1	0.17	

TABLE 5

POTASSIUM NITRATE
Chi-Square Analyses
% Mortality

Dose mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	37.141*	-
10.0	0.05	0.244	0.505	0.097
20.0	0.03	8.833*	0.00	0.263
30.0	1.975	-	-	-
40.0	5.591*	107.418*	0.339	0.144
80.0	0.447	125.571*	0.007	0.144
88.0	-	86.229*	-	-
100.0	0.103	-	6.929*	-
160.0	-	154.325*	-	-
175.0	-	115.561*	-	-
200.0	0.166	-	0.318	1.156
320.0	2.02	270.725*	2.883	0.012
350.0	-	178.426*	-	-
500.0	16.115*	-	-	-
700.0		72.76*	-	-
1000.0	33.108*	-	-	-
All doses (DF)	211.42*(10)	497.55*(10)	79.13*(8)	5.074(6)

*Probability < 0.05 - 0.005

TABLE 6

POTASSIUM NITRATE
LINEAR REGRESSION of
LOG DOSE AGAINST PROBIT OF MORTALITY

	Air Cell	
	0 hrs	96 hrs
LD-30 (Range) mg/kg	424(526-319)	34(64-8)
LD-50 (Range) mg/kg	569(775-460)	73(151-33)
LD-70 (Range) mg/kg	762(1261-602)	159(563-85)
LD-90 (Range) mg/kg	1164(2710-832)	488(5776-211)

TABLE 7

POTASSIUM NITRATE
Chi-Square Analyses
Abnormalities

Dose mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	0.212	-
10.0	0.026	0.00	0.33	0.309
20.0	0.326	0.007	0.023	0.0038
30.0	19.482*	-	-	-
40.0	0.211	0.00	0.697	0.536
80.0	0.032	0.00	0.024	0.038
88.0	-	0.00	-	-
100.0	3.511	-	0.00	-
160.0	-	0.00	-	-
175.0	-	0.00	-	-
200.0	21.631*	-	0.225	0.846
320.0	0.034	0.00	0.00	0.067
350.0	-	0.00	-	-
500.0	1.424	-	-	-
1000.0	1.331	-	-	-
All doses (DF)	109.5(10)*	4.985(9)	3.65(8)	4.087(6)

*Probability $< 0.05 - 0.005$

TABLE 8

POTASSIUM NITRATE
Chi-Square Analyses
Head-Limbs-Skeletal-Visceral Abnormalities

Dose mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
1.0	-	-	0.21	-
10.0	0.34	0.00	0.33	0.31
20.0	0.01	0.01	0.00	0.31
30.0	0.56	-	-	-
40.0	0.21	0.00	0.19	0.31
80.0	0.03	0.00	0.00	0.31
88.0	-	0.00	-	-
100.0	3.51	-	0.00	-
160.0	-	0.00	-	-
175.0	-	0.00	-	-
200.0	0.00	-	0.23	0.85
320.0	0.03	0.00	0.00	0.07
350.0	-	0.00	-	-
500.0	1.42	-	-	-
1000.0	1.33	-	-	-
All doses (DF)	5.97(10)	4.99(9)	6.65(8)	0.99(6)

(sheet 1 of 3 sheets)

TABLE 9

POTASSIUM NITRATE IN WATER

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
Untreated controls	565	6	1	anophthalmia of eye
			1	anophthalmia - bilateral, encephalocele, agenesis-maxilla, celosomia
			1	exencephaly
			1	cachexia, anophthalmia-bilateral
			1	cachexia.
			1	ankylosis - rt.
Drilled control - 0 hrs	184	1	1	celosomia
Drilled control - 96 hrs	222	0	0	
Pierced control - 0 hrs	80	2	1	anophthalmia-bilateral, dysgnathia
			1	anophthalmia-lf., dysgnathia
Pierced control - 0 hrs	240	2	1	excencephaly
			1	anophthalmia-bilateral, dysgnathia
AIR CELL, 0 HRS				
1,000.0 mg/kg	20	0	0	
500.0	19	0	0	
320.0	99	0	0	
200.0	45	9	1	anophthalmia-lf., dysgnathia
			1	fibrosis-liver
			1	fibrosis-liver,granulation tissue-renal tubule
			1	vacuolization-liver
			3	vacuolization-liver, granulation tissue - liver
			1	granulation tissue - liver
			1	agenesis - liver
100.0	9	0	0	
80.0	100	0	0	
40.0	99	1	1	anophthalmia - rt., dysgnathia

TABLE 9, cont'd
(sheet 2 of 3 sheets)

POTASSIUM NITRATE IN WATER

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	TERATOGENIC FINDINGS SPECIFIC FINDINGS NO.	DESCRIPTION
AIR CELL, 0 HRS cont'd				
20.0	117	1	1	hypopigmentation
10.0	119	2	1	ataxia
			1	cachexia
0.0	241	1	1	anophthalmia-rt., dysgnathia
AIR CELL 96 HRS				
700.0 mg/kg	27	0	0	
480.0	64	0	0	
400.0	64	0	0	
350.0	27	0	0	
320.0	115	0	0	
175.0	27	0	0	
160.0	50	0	0	
88.0	27	0	0	
80.0	115	0	0	
40.0	115	0	0	
20.0	142	1	1	celosomia
10.0	65	0	0	
0.0	221	0	0	

TABLE 9 cont'd
(sheet 3 of 3 sheets)

POTASSIUM NITRATE IN WATER

TERATOGENIC FINDINGS

TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS NO.	DESCRIPTION
YOLK, 0 HRS				
320.0 mg/kg	10	0	0	
200.0	144	2	1	anophthalmia-bilateral, agenesis-maxilla
			1	anophthalmia-rt., dysgnathia
100.0	13	0	0	
80.0	139	1	1	hypopigmentation
40.0	152	3	2	celosomia
			1	cachexia
20.0	138	1	1	dwarfism
10.0	124	2	1	celosomia
			1	dysgnathia
1.0	42	1	1	dysgnathia
0.0	140	0	0	
YOLK - 96 HRS				
320.0 mg/kg	90	0	0	
200.0	29	9	9	
80.0	120	2	1	microphthalmia - bilateral, exencephaly
			1	hydrocephalus
40.0	120	3	1	cachexia
			1	exencephaly
			1	acrania
20.0	120	2	1	exencephaly
			1	hemorrhage - head

Table 10

POTASSIUM NITRATE

POST HATCH DATA

Injection Date 2/29/72

Label	Dose mg/kg	Average Age at Sexual Maturity	Average Body Weights, gm				Average Feed Cons./Bird	
			at Hatch	6 wks		6 mo		6 wks, gm	6 mo kg
				M	F	M	F		
222	0 (H_2O)	138	38.0	456	406	1703	1589	1002	11.0
223	10.0	141	36.5	429	414	1703	1816	958	12.2
227	320.0	140	36.0	455	409	1648	1589	1043	10.8